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Safety and efficacy of manganese hydroxychloride as feed additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

The additive under assessment, manganese hydroxychloride, contains $\geq$ 45% manganese (Mn). Manganese hydroxychloride is a safe source of manganese for all target species when used up to the maximum level of total manganese in complete feed authorised in the EU (*Fish* 100 (total) and *Other species* 150 (total) mg Mn/kg feedingstuffs). Manganese hydroxychloride would substitute for other manganese sources in feed. It does not differently affect tissue deposition compared to manganese sulphate. The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that the use of manganese hydroxychloride in animal nutrition is safe for the consumers, provided the maximum authorised manganese content in complete feed is respected. In the absence of specific data, the FEEDAP Panel cannot conclude on the safety for the user when handling the additive. The use of manganese hydroxychloride in animal nutrition for all animal species is not expected to pose a risk to the environment. Manganese hydroxychloride is considered a source of bioavailable manganese as indicated by a dose-dependent increase of manganese deposition in bone and liver based on two studies on chickens for fattening. The FEEDAP Panel concludes that the additive is efficacious in meeting the manganese requirements for all animal species/categories.

Keywords: nutritional additive, compounds of trace elements, manganese, manganese hydroxychloride, safety, efficacy

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of manganese hydroxychloride for all animal species.

Manganese (Mn), an essential trace element, functions as an enzyme activator and is a constituent of several enzymes, e.g. glycosyl transferases, pyruvate carboxylase, manganese superoxide dismutase. Primary signs of manganese deficiency are impaired growth, skeletal abnormalities, depressed reproductive function, ataxia of the newborn and faults in lipid and carbohydrate metabolism.

The additive under assessment, manganese hydroxychloride (tribasic manganese chloride, TBMC), contains $\geq$ 45% manganese.

Manganese hydroxychloride is a safe source of manganese for all target species when used up to the maximum level of total manganese in complete feed authorised in the EU (*Fish* 100 (total) and *Other species* 150 (total) mg Mn/kg feedingstuffs).

Manganese hydroxychloride would substitute for other manganese sources in feed. It does not differently affect tissue deposition compared to manganese sulphate. The FEEDAP Panel concludes that the use of TBMC in animal nutrition is safe for the consumers, provided the maximum authorised manganese content in complete feed is respected.

In the absence of specific data, the FEEDAP Panel cannot conclude on the safety for the user when handling manganese hydroxychloride.

The use of manganese hydroxychloride in animal nutrition for all animal species is not expected to pose a risk to the environment.

TBMC is considered a source of bioavailable manganese as indicated by a dose-dependent increase of manganese deposition in bone and liver based on two studies on chickens for fattening. The FEEDAP Panel concludes that the additive is efficacious in meeting the manganese requirements for all animal species/categories.

The FEEDAP Panel made some recommendations concerning the name and specifications of the additive.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Micronutrients[2] for authorisation of manganese hydroxychloride (IntelliBond® M), when used as a feed additive for all animal species (category: nutritional additives; functional group: compounds of trace elements).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 30 September 2013.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product manganese hydroxychloride (IntelliBond® M), when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

Manganese (Mn) is an abundant element which makes up about 0.1% of the Earth's crust. It exists in a variety of oxidation states, Mn^{2+} and Mn^{3+} being the most biologically important. The elemental (metal) form of manganese does not occur naturally in the environment; however, manganese is a component of over 100 minerals (ATSDR, 2012).

The EFSA Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA Panel, 2013) summarised the biological functions of manganese as follows: "Manganese is an essential dietary mineral for mammals; it is a component of metalloenzymes such as superoxide dismutase, arginase and pyruvate carboxylase, and is involved in amino acid, lipid and carbohydrate metabolism (EC, 1993; IOM, 2001; NHMRC, 2006). Glycosyltransferases and xylosyltransferases, which are involved in proteoglycan synthesis (e.g. for bone formation), are sensitive to manganese status in animals (Nielsen, 1999)". Primary manifestations of manganese deficiency in livestock are impaired growth, skeletal abnormalities, depressed reproductive function, ataxia of the newborn and faults in lipid and carbohydrate metabolism (Suttle, 2010). ATSDR (2012) and, more recently, Lucchini et al. (2015) reviewed the absorption, distribution, metabolism and excretion (ADME) and toxicity of manganese.

The additive manganese hydroxychloride has not been previously authorised in the European Union (EU). However, other manganese compounds are authorised to be used as nutritional feed additives (trace elements) in the EU by Commission Regulation (EC) No 1334/2003[3] and amendments.

The Scientific Committee on Animal Nutrition (SCAN) issued a report on the use of manganomanganic oxide in feedingstuffs (EC, 2002). EFSA issued an opinion on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005) and three opinions on a manganese chelate of hydroxy analogue of methionine (EFSA, 2008a; EFSA FEEDAP Panel, 2009, 2010). In the frame of re-evaluation, EFSA has delivered four opinions on manganese-based additives including: manganous chloride, tetrahydrate; manganous oxide; manganous sulphate monohydrate; manganese chelate of amino acids, hydrate; manganese chelate of glycine, hydrate (EFSA FEEDAP Panel, 2013a,b,c, 2016).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

[2] Micronutrients USA LLC. This applicant is represented by Regal B.V., Wilhelminalaan 90, 6042 EP, Roermond, The Netherlands.

[3] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of manganese hydroxychloride as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[5] and the applicable EFSA guidance documents.

The Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, scientific papers and other scientific reports, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of manganese hydroxychloride. The Executive Summary of the EURL report can be found in the Annex.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and efficacy of manganese hydroxychloride is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

3. Assessment

The applicant is seeking authorisation for the use of manganese hydroxychloride in feed for all animal species/categories. This manganese compound is not currently authorised in the EU as feed additive. Manganese hydroxychloride, a specific inorganic manganese source, is applied as a nutritional additive, functional group 'Compounds of trace elements', to be used in feedingstuffs for all species up to maximum manganese levels in complete feeds authorised in the EU.

3.1. Characterisation

For the purpose of this opinion, 'manganese hydroxychloride' is the compound of manganese intended to release the element for its nutritional function in the organism. The compound is further processed during manufacturing to produce a formulated additive, which is referred to as the 'additive' throughout the scientific opinion.

3.1.1. Manufacturing process[7]

The manufacturing process of the product is fully described in the technical dossier.

3.1.2. Characterisation of the additive

The compound 'Manganese hydroxychloride' is identified by the Chemical Abstracts Service (CAS) No 39438-40-9. Its molecular formula is $Mn_2(OH)_3Cl$ and it has a molecular weight of 196.35 Da. Other names are tribasic manganese chloride (TBMC) and basic manganese chloride. The theoretical content of manganese is 56%.

[4] FEED dossier reference: FAD-2012-0040.
[5] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.
[6] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2012-0040_MnOHCl.doc_.pdf
[7] This section has been amended following the confidentiality claims made by the applicant.

Specifications of the additive are for manganese > 44%, for chlorine 16–19% and for moisture < 2%.[8] Analytical data of the additive were provided for two sets of five batches each.[9,10] They showed an average manganese content of 45.9% (range: 45.2–46.8%) for the first data set and 45.6% (range: 44.9–46.1%) for the second data set. The chlorine content provided for the second data set only was on average 14.0% (range: 13.4–14.4%, below the specification range). The content of moisture ranged between 0.4% and 0.8% for the first data set and from 0.3% to 1.0% for the second data set. By X-ray diffraction (XRD) analysis of one batch, the additive contains 72.2% of TBMC.[11] As this content of TBMC would provide only 40.4% manganese, which is below the average analytical concentration (45.8%), it can reasonably be assumed that the additive also contains manganous oxide, involved in the production process, in the order of approximately 7%.

Impurities resulting from the starting material of manufacturing were measured in the second set of five batches by XRD.[10] Mean values for the major impurities (expressed as oxides) were (in descending order of quantity): iron 5.42%, aluminium 4.84%, silicon 1.93%, potassium 0.89%, sodium 0.65%, calcium 0.28%; the rest of analysed impurities (including barium, magnesium, cobalt, chromium, copper, molybdenum, nickel, titanium and zinc; also expressed as oxides) summed in their average levels up to 1.3%. Because of possible concerns about nickel content, the applicant was requested to provide specific data; the nickel content was found in the range of 0.03–0.04%.[12]

Levels of heavy metals, arsenic and fluorine were analysed in the first set of five batches;[9] the results reported were: cadmium 0.69–0.82 mg/kg, lead 26–29 mg/kg, arsenic 28–30 mg/kg and fluorine 2.6–4.1 mg/kg.[13] Dioxins and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) were measured in three batches; the results showed 0.091–0.127 ng WHO-PCDD/F-TEQ)/kg[14] and 0.128–0.139 ng WHO-PCDD/F-PCB-TEQ/kg.[9] All the reported values are within limits set in the Directive 2002/32/EC on Undesirable Substances in animal feed[15] for feed additives belonging to the functional group compounds of trace elements or, where no specific limit is mentioned, do not represent a safety concern.

The additive is an olive green to light brown granular powder with no odour. Its bulk density is 830 kg/m^3; tapped density is 937 kg/m^3.[9] The solubility in water is low.[8]

Mean particle size was measured by sieve analysis in five batches and calculated to be in the range of 221–238 μm.[9] Three of those batches were also analysed by laser diffraction; no particles below 90 μm diameter were identified.[16]

The dusting potential of the additive (three batches) was measured by the Stauber–Heubach method. The calculated average was 95 mg/m^3 (range: 75–110).[17]

3.1.3. Stability and homogeneity

For inorganic compounds of trace elements, stability studies are generally not required.

The capacity of the additive to be homogeneously distributed in feed was tested in feed for laying hens (background manganese content in the unsupplemented feed was 24 mg/kg).[18] A total of 100 mg Mn from TBMC/kg complete feed was mixed into the feed. Subsequently, 15 subsamples of the feed were analysed for total manganese content. The calculated coefficient of variation was 7.5% of total Mn (111 mg Mn/kg).

3.1.4. Conditions of use

The additive is intended to be incorporated directly to feedingstuffs or via premixtures for all animal species/categories up to the total maximum manganese content authorised in complete feeds in the EU: Fish 100 (total) and Other species 150 (total) mg/kg feedingstuffs.

[8] Technical Dossier/Section II/Annex 2.1.3.a.
[9] Technical Dossier/Section II/Annex 2.1.3.b.
[10] Technical Dossier/Supplementary information August 2015/Appendix 3-CoA-IBM-Characterization.pdf.
[11] Technical Dossier/Supplementary information August 2015/Appendix 4-XRD-IBM-0039.pdf.
[12] Technical dossier/Supplementary information October 2015/Annex 1 IBM-Ni-EFSA-10-14-2015.pdf.
[13] The FEEDAP Panel notes that the specification set in the dossier for Cd (< 30) and As (< 100) is considerably above the legal maximum allowed for compounds of trace elements.
[14] Technical dossier/Supplementary information June 2015/Annex 2.
[15] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[16] Technical dossier/Section II/Annex 2.1.5.a.
[17] Technical dossier/Section II/Annex 2.4.3.a.
[18] Technical dossier/Section II/Annex 2.4.2.a.

3.2. Safety

3.2.1. Safety for the target species

The maximum tolerable levels and requirements for manganese have been reviewed by the FEEDAP Panel in previous opinions (e.g. EFSA FEEDAP Panel, 2016).

3.2.1.1. Tolerance studies with chickens for fattening

The applicant has provided five studies to support the safety of the additive.

Four studies on chickens for fattening, two long-term[19] and two short-term (Conly et al., 2012), were submitted initially by the applicant. These studies were not considered as tolerance studies because essential end-points (haematology, clinical chemistry, gross pathology) required for these studies in the respective Guidelines (Regulation (EC) No 429/2008) and EFSA Guidance for tolerance and efficacy were not examined; two studies were below the minimum duration of tolerance studies. Upon request by EFSA, the applicant submitted a fifth study meeting the requirements for tolerance studies.

A total of six hundred and forty 1-day-old male chickens for fattening (Cobb 500) were divided into eight groups with 10 pens, each pen with eight birds (80 chickens per treatment).[20] The treatments comprised 60 —dose representing requirements for chickens for fattening, according to NRC, 1994—, 120, 960 and 1,920 (maximum tolerable levels (MTL) for poultry is 2,000 mg Mn/kg feed, according to NRC 2005) mg supplemental Mn/kg complete feed from either manganese sulphate ($MnSO_4$) or the additive under assessment. The total manganese concentrations were analysed in both starter and grower diets (see Table 1). Birds had *ad libitum* access to water and feed for a period of 35 days. The diets were based on maize–soya bean meal; the starter diet was given during the first 21 days, followed by the grower diet until study completion. After 35 days, blood samples (haematology[21] and blood chemistry[22]) were obtained from eight birds per treatment. These birds were killed for macroscopic inspection, and tissue sampling. Manganese content was investigated in bone (tibiotarsus) as a biomarker of manganese bioavailability (see EFSA, 2008a), as well as in edible tissues (breast muscle and liver, see Table 1).

The pen was considered as the statistical unit. Prior to statistical analysis, an outlier test (Grubb's test) was conducted and one pen from each of treatment groups TBMC 60, 120 and 1,920 mg/kg were removed from the statistical analysis for feed consumption and feed/gain ratio. The data set was analysed by a bi-factorial ANOVA taking into account the effects of manganese source and supplementation level, as well as their interaction. Levels of each main factor were compared using Tukey test (Tukey–Kramer in case of different number of replicates in the treatments due to removed outliers).

No differences in performance parameters (average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F/G)) were observed between groups with the exception of birds receiving TBMC at the highest level, which ate and grew less than the birds from other treatments (see Table 1). At the highest supplemented TBMC level, mortality was numerically higher (no statistics provided); feed intake and weight gain were significantly decreased and F/G ratio was significantly increased (Table 1). At the highest supplemental level, $MnSO_4$ did not affect significantly any of these parameters. A dose-dependent increase of the manganese concentration in bone was observed for both manganese sources, showing a comparable bioavailability, albeit values were slightly lower for TBMC at the two higher dose levels. No significant effects of manganese supplementations were seen in the breast muscle, but the two highest manganese doses (960 and 1,920 mg/kg) increased significantly the manganese concentration in liver (Table 1).

[19] Technical dossier/Section III/Annex 3.1.1.a.
[20] Technical Dossier/Supplementary information June 2015/Annex 4 14-29 TBMC tolerance study.
[21] Hematocrit, erythrocytes, white blood cells (WBCs), heterophils, eosinophils, basophils, lymphocytes, monocytes.
[22] Alkaline phosphatase (AP), aspartate aminotransferase (AST); glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), amylase, creatine kinase (CK), lipase, cholesterine, triglyceride, uric acid, sodium, potassium, calcium, phosphates, bile acids, albumin, protein.

Table 1: Zootechnical parameters and manganese tissue deposition in chickens for fattening measured at 35 days

Manganese source	$MnSO_4$				TBMC			
Mn in feed (mg/kg)								
Added (intended)	60	120	960	1,920	60	120	960	1,920
Total (analysed)	90[(a)]/71[(b)]	134/170	980/950	1,875/1,900	86/96	174/140	933/1,000	1,930/1,600
Mortality (%)	3.8	5.0	2.5	3.8	1.4	2.9	0.0	10.0
ADFI (g)[(c)]	106.9	106.3	104.9	106.3	105.8	107.3	106.0	81.0
ADG (g)[(c)]	74.3	74.6	72.8	73.3	73.1	74.1	72.6	49.8
F/G Ratio[(c)]	1.44	1.42	1.44	1.45	1.45	1.45	1.46	1.63
Mn in tissues (mg/kg crude ash for bone; mg/kg dry matter for breast muscle and liver)								
Bone (tibiotarsal)[(d)]	15	16	43	72	13	18	36	49
Breast muscle[(e)]	1.9	1.3	1.3	2.3	1.0	0.9	1.2	1.4
Liver[(f)]	10.9	9.9	14.9	17.9	10.1	9.7	14.7	15.4

ADFI: average daily feed intake; ADG: average daily gain; F/G: feed to gain ratio; Mn: manganese; $MnSO_4$: manganese sulphate; TBMC: tribasic manganese chloride.
(a): Starter (1–21 days).
(b): Grower (22–35 days).
(c): Significant differences ($p < 0.001$) for manganese sources, levels and the interaction source × level; significant difference ($p < 0.001$) between the highest manganese level and the three lower levels.
(d): Significant differences ($p < 0.001$) for manganese sources, levels and the interaction source × level.
(e): Significant difference ($p < 0.05$) for manganese sources.
(f): Significant differences ($p < 0.001$) between the two lower and the two higher manganese levels.

Macroscopic inspection of organs did not reveal any treatment-related changes. Haematology was done in two different laboratories resulting, at least partially, in highly different values (e.g. mean WBC count ($n = 32$) for Lab1: 7.1 and for Lab2: $1.2 \times 10^3/\mu L$); the statistical evaluation was based on merged values. The haematological data could therefore not be further assessed.

Several serum enzymes (AP, AST, GLDH, LDH and CK) showed a significant reduction in the highest TBMC group as compared to the corresponding level of $MnSO_4$ and to lower manganese levels from TBMC. Serum potassium showed a mean concentration of 6.9 mmol/L (range: 6.4–7.7) which is above physiological levels reported not to exceed 5 mmol/L for this age class in male chickens for fattening (Bowes et al., 1989). The FEEDAP Panel considers that the observed enzyme changes do not indicate *per se* an adverse effect.

The study showed that in total TBMC is less safe to chickens for fattening compared to the standard compound of manganese, the $MnSO_4$, at the highest concentration tested which is near to the MTL for poultry (NRC, 2005). At lower concentrations, no differences between the two manganese sources were seen considering zootechnical and clinical chemistry end-points.

3.2.1.2. Conclusions on the safety for target species

Based on an adequate tolerance study on chickens for fattening, the FEEDAP Panel concludes that manganese hydroxychloride is a safe source of manganese when used up to the maximum content of total manganese in complete feed authorised in the EU.

3.2.2. Safety for the consumer

In its previous opinions in the context of the re-evaluation of manganese compounds as nutritional feed additives, the FEEDAP Panel has reviewed the metabolic and toxicological profile of manganese, consumer exposure and its safety (see e.g. EFSA FEEDAP Panel, 2016); the Panel concluded that the use of the manganese compounds assessed in animal nutrition is of no concern for the safety of consumers, provided that the current maximum total contents of manganese authorised in feed are respected. Consequently, only results and conclusions concerning the additive under assessment are reported below.

3.2.2.1. Deposition studies

The deposition of manganese in breast muscle and liver was investigated in chickens for fattening fed either TBMC or $MnSO_4$ at doses of 60, 120, 960 and 1,920 mg Mn/kg feed. The experimental design is described in Section 3.2.1. Results are shown in Table 1.

The manganese content in breast muscle varied for the eight experimental groups between 0.9 and 2.3 mg/kg dry matter (DM). However, no significant differences could be identified between the manganese sources and the different supplementation levels.

No differences were seen between the manganese sources for manganese deposition in liver. The two higher supplementation rates resulted, independent of the manganese source, in significantly higher manganese concentrations than the two lower supplementation levels.

Overall, the results show that manganese deposition in muscle and liver of chickens for fattening does not increase at supplementation levels compatible with the maximum authorised level in complete feed in the EU. Moreover, no differences between the TBMC and $MnSO_4$ could be expected considering manganese deposition in chickens for fattening. The FEEDAP Panel notes that, albeit no data were provided on the deposition of manganese from TBMC in milk and eggs, the available literature indicates that (i) no differences could be expected concerning manganese deposition in eggs between organic and inorganic manganese sources (Mabe et al., 2003; Huyghebaert et al., 2006; Dobrzanski et al., 2008) and (ii) the deposition of manganese from inorganic sources in those edible products occurs only to a minor extent (EPA, 2003; Santos et al., 2004; Leblanc et al., 2005; Van Overmeire et al., 2006; Souci et al., 2008).

3.2.2.2. Conclusions on safety for consumers

Tissues and products of animal origin generally provide a low contribution to the overall manganese dietary intake; however, feed supplementation should not increase significantly the oral exposure of consumers by promoting manganese deposition in edible tissues and products.

As manganese hydroxychloride would substitute for other manganese sources in feed and there was no difference in tissue deposition between manganese hydroxychloride and $MnSO_4$, an increased manganese exposure of consumers due to the use of manganese hydroxychloride as feed additive is not expected, provided the maximum authorised manganese content in complete feed is respected. The FEEDAP Panel concludes that the use of manganese hydroxychloride in animal nutrition is safe for the consumers.

3.2.3. Safety for the user

No specific studies relevant to user safety conducted with the additive were provided.

No studies on irritancy to skin and eyes and skin sensitisation with TBMC were provided. The material safety data sheet[23] indicates that metal chloride compounds, such as TBMC, are likely to be irritant to skin and eyes, which may be induced by an allergic reaction.

Manganese is a recognised workplace toxicant upon inhalation exposure (ATSDR, 2012). The data available to estimate inhalation exposure indicate high particle size (no particles below 90 μm) and a dusting potential of about 95 mg Mn/m^3. These data suggest a certain potential for inhalation exposure. However, the dust particle size and the manganese content of the dust were not determined. Consequently the Panel is not in the position to conclude on user safety upon inhalation.

In view of the above, the FEEDAP Panel cannot conclude on the safety for the user when handling manganese hydroxychloride.

3.2.4. Safety for the environment

In its previous opinions in the context of the re-evaluation of manganese compounds as nutritional feed additives, the FEEDAP Panel has reviewed the safety for the environment on various manganese compounds and concluded that the use of the said compounds in animal nutrition for all animal species is not expected to pose a risk to the environment (see e.g. EFSA FEEDAP Panel, 2016). The substitutive use of manganese hydroxychloride for other manganese compounds would not essentially change the above conclusion.

3.3. Efficacy

To support efficacy, the applicant submitted a short-term study with chicken for fattening and compared the bioavailability of TBMC with $MnSO_4$ (Conly et al., 2012). Moreover, the tolerance study (see Section 3.2.1) provided some data supporting efficacy of the additive.

[23] Technical Dossier/Section II/Annex 2.5.2.a.

A total of two hundred and fifty-two 1-day-old chickens for fattening (Cobb × Cobb, both sexes) were randomly assigned to seven treatments (6 pens/treatment; 6 birds/pen) and fed for 21 days with a maize–soya bean meal-based diet. The basal diet contained 45 mg Mn/kg feed and was supplemented with 30, 60 or 130 mg Mn/kg from TBMC or $MnSO_4$. The analysed manganese content of all experimental groups is shown in Table 2. The basal diet was fed to all pens for 9 days to promote maximal absorption of manganese followed by 12 days of the assigned treatment diets (Table 2). Body weight gain and feed intake were measured. All birds were killed at 21 days for tissue sample collection (tibia, liver). Tissue samples were taken from two birds from each pen for mineral analysis by inductively coupled plasma (ICP) analysis; values within pen were averaged for statistical analysis.

Table 2: Manganese content of feed, feed intake, body weight and manganese concentration in tibia and liver of chicken for fattening (initial body weight: 48 g/chicken)

Item	Basal diet	$MnSO_4$			TBMC		
Intended supplemented Mn (mg/kg feed)	–	30	60	130	30	60	130
Total Mn analysed (mg/kg feed)	45	70	98	184	79	109	183
Body weight gain (g). Day 10–21	492	516	508	504	510	496	513
Feed intake (g/chicken). Day 10–21	865	845	816	801	859	804	872
Mn content (mg/kg ash)							
Tibia	5.3^{c}	5.8^{bc}	6.5^{b}	8.5^{a}	5.8^{bc}	6.6^{b}	8.2^{a}
Liver(*)	8.6^{bc}	8.4^{c}	9.7^{a}	9.9^{a}	8.2^{c}	9.5^{ab}	10.0^{a}

Mn: manganese; $MnSO_4$: manganese sulphate; TBMC: tribasic manganese chloride.
a, b, c: Different superscripts within a row indicate statistical differences ($p < 0.05$).
(*): Units expressed in the publication as 'ppm on ash-weight basis'; however, comparing with other literature values and also considering additional data given for liver 'ash' in the same publication, the FEEDAP Panel has doubts on the correctness of the given dimension and considers it more plausible that the values refer to dry matter weight.

Feed intake and body weight gain were not significantly influenced by manganese sources and levels. Manganese doses, but not source, had a significant influence of manganese content of tibia and liver (Table 2).

3.3.1. Conclusions on efficacy for target species

Manganese hydroxychloride is considered a bioavailable source of manganese as indicated by a dose-dependent increase of manganese in bone at use levels in a short-term experiment and, at higher levels, in the long-term tolerance study. This conclusion is supported by similar findings on the manganese content of liver in both studies.

3.4. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[24] and good manufacturing practice.

4. Conclusions

Manganese hydroxychloride is a safe source of manganese for all target species when used up to the maximum level of total manganese in complete feed authorised in the EU (*Fish* 100 (total) and *Other species* 150 (total) mg Mn/kg feedingstuffs).

Manganese hydroxychloride would substitute for other manganese sources in feed. It does not differently affect tissue deposition compared to $MnSO_4$. The FEEDAP Panel concludes that the use of manganese hydroxychloride in animal nutrition is safe for the consumers, provided the maximum authorised manganese content in complete feed is respected.

In the absence of specific data, the FEEDAP Panel cannot conclude on the safety for the user when handling manganese hydroxychloride.

[24] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

The use of manganese hydroxychloride in animal nutrition for all animal species is not expected to pose a risk to the environment.

Manganese hydroxychloride is considered a source of bioavailable manganese as indicated by a dose-dependent increase of manganese deposition in bone and liver based on two studies on chickens for fattening. The FEEDAP Panel concludes that the additive is efficacious in meeting the manganese requirements for all animal species/categories.

5. Recommendations

In analogy with the authorisation of di copper chloride tri hydroxide, the FEEDAP Panel proposes 'Di manganese chloride tri hydroxide' as the name of the compound, to differentiate from other manganese chloride hydroxides.

Specifications for cadmium and arsenic should be amended to comply with Directive 2002/32/EC on Undesirable substances in animal feed. The maximum content of manganous oxide should also be specified.

Documentation provided to EFSA

1) Dossier Manganese hydroxychloride (IntelliBond® M). October 2012. Submitted by Feedtest.
2) Dossier Manganese hydroxychloride (IntelliBond® M). Supplementary information. June 2015. Submitted by Feedtest.
3) Dossier Manganese hydroxychloride (IntelliBond® M). Supplementary information. August 2015. Submitted by Feedtest.
4) Dossier Manganese hydroxychloride (IntelliBond® M). Supplementary information. October 2015. Submitted by Feedtest.
5) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Manganese hydroxychloride.
6) Comments from Member States.

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Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ADME	absorption, distribution, metabolism and excretion
AP	alkaline phosphatase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
CAS	Chemical Abstracts Service
CK	creatine kinase
DM	dry matter
EC	European Commission
EPA	Environmental Protection Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
F/G	feed to gain ratio
GLDH	glutamate dehydrogenase
GMO	genetically modified organism
ICP	inductively coupled plasma
IOM	Institute of Medicine
LDH	lactate dehydrogenase
Mn	manganese
$MnSO_4$	manganese sulphate
MTL	maximum tolerable level
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NHMRC	National Health and Medical Research Council
NRC	National Research Council
PCBs	polychlorinated biphenyls
PCDD	polychlorinated dibenzodioxin
SCAN	Scientific Committee on Animal Nutrition
TBMC	tribasic manganese chloride
TEQ	toxic equivalent factor
WBC	white blood cell
WHO	World Health Organization
XRD	X-ray diffraction

Annex – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for manganese hydroxychloride

In the current application authorisation is sought under article 4(1) for *manganese hydroxychloride* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all species and categories. *Manganese hydroxychloride* ($Mn_2(OH)_3Cl$) is a brown free-flowing crystalline powder with a minimum content of 44% *total manganese* and a *chloride* content ranging from 16 to 19%. The *feed additive* is intended to be mixed into *premixtures* and *feedingstuffs*. The Applicant suggested maximum levels ranging from 100 to 150 mg *total manganese* /kg *feedingstuffs*.

For the quantification of *chloride* in the *feed additive*, the Applicant suggested the ion chromatography method described in the U.S. Environmental Protection Agency (EPA method 300.0, Rev.2.1). However, the EURL recommends instead the Community method, based on titration, for determination of *chlorine* in the *feed additive*.

For the quantification of *total manganese* content in the *feed additive, premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated CEN method (EN 15510) based on Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). In addition to this method, the EURL already recommended - in the frame of the evaluation of the manganese group (cf. FAD-2010-1169, FAD-2010-0088 and FAD-2010-0235) - two other ring-trial validated methods: EN 15621, based on ICP-AES after pressure digestion; and - EN ISO 6869 based on Atomic Absorption Spectrometry (AAS), together with the Community method, further ring-trial validated by the UK Food Standards Agency.

Based on the acceptable method performance characteristics available the EURL recommends for official control all the methods mentioned above for the quantification of *total manganese* in the *feed additive, premixtures* and *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.